PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07J 41/00, A61K 31/575, C07J 43/00

A1

(11) International Publication Number: **WO 99/45024**

(43) International Publication Date: 10 September 1999 (10.09.99)

(21) International Application Number: PCT/GB99/00681

(22) International Filing Date: 8 March 1999 (08.03.99)

(30) Priority Data:
9804861.4 6 March 1998 (06.03.98) GB

(71) Applicant *(for GB only)*: MARSDEN, John, Christopher [GB/GB]; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).

(71) Applicant *(for all designated States except US)*: RESEARCH INSTITUTE FOR MEDICINE AND CHEMISTRY INC. [US/US]; 49 Amherst Street, Cambridge, MA 02142 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HESSE, Robert, Henry [US/US]; 6 Sargent Road, Winchester, MA 01890 (US). SETTY, Sundara, Katugam, Srinivasasetty [IN/US]; 402 Ringe Avenue, Cambridge, MA 02140 (US). RAMGOPAL, Malathi [IN/US]; 8 Garfield Lane East, Andover, MA 01810 (US).

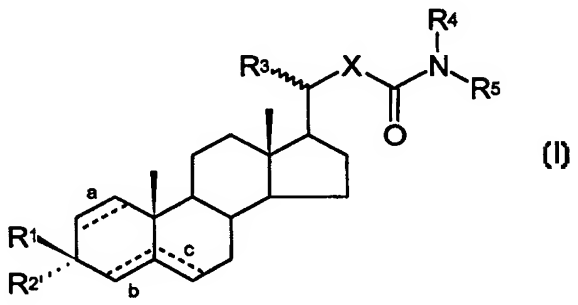
(74) Agents: MARSDEN, John, Christopher et al.; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims.

(54) Title: CHOLENIC ACID AMIDES AND PHARMACEUTICAL COMPOSITIONS THEREOF

(57) Abstract



(I)

Novel sterol derivatives of formula (I), in which: R^1 represents a hydroxyl group or protected hydroxyl group, R^2 represents a hydrogen atom and a double bond is present at c, or R^1 and R^2 together represent an oxo group and a double bond is present at b or double bonds are present at a and b; R^3 represents a methyl group having α– or β–configuration; R^4 and R^5, which may be the same or different, are selected from hydrogen atoms and aliphatic, cycloaliphatic, araliphatic and aryl groups, or togheter with the nitrogen atom to which they are attached form a heterocyclic group; and X represents a polymethylene group containing 2–5 carbon atoms, an oxa group–containing analogue thereof in which a methylene group other than that attached to the $-CO.NR^4R^5$ moiety is replaced by an oxygen atom, or an unsaturated analogue thereof containing up to two double bonds. Active compounds of the invention exhibit potent effects on the modulation of cell growth and differentiation and possess an advantageous therapeutic ratio by virtue of their low levels of calcaemic activity.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

CHOLENIC ACID AMIDES AND PHARMACEUTICAL COMPOSITIONS THEREOF

This invention relates to novel sterol derivatives, more particularly to sterol derivatives in which the 17-position side chain terminates in an amide group and which exhibit cell modulating activity.

It is well known that 9,10-seco sterol derivatives such as vitamin D_3 play a vital role in the metabolism of calcium by promoting intestinal absorption of calcium and phosphorus, maintaining adequate serum levels of calcium and phosphorus, and stimulating mobilisation of calcium from the bone fluid compartment in the presence of parathyroid hormone. Following the discovery that D vitamins are hydroxylated *in vivo*, at the 25-position in the liver and at the 1α-position in the kidneys, and that the resulting 1α,25-dihydroxy metabolite is the biologically active material, extensive studies have been carried out on vitamin D analogues hydroxylated at, for example, the 1α- and 24R- or 25-positions.

The natural metabolite 1α,25-dihydroxy vitamin D_3 has additionally been found to have effects on cellular metabolism, these cell modulating effects including stimulation of cell maturation and differentiation, immunosuppressive effects and immunopotentiating effects (e.g. by stimulating the production of bactericidal oxygen metabolites and the chemotactic response of leukocytes). However, the potent effects of compounds such as 1α,25-dihydroxy vitamin D_3 on calcium metabolism will normally preclude their use in this area, since doses sufficient to elicit a desired cell modulating effect will tend to lead to unacceptable hypercalcaemia.

This has led to attempts to synthesize new vitamin D analogues which have reduced effects on calcium metabolism but which still exhibit the desired effects on cellular metabolism. Representative examples of such analogues, together with summaries of earlier attempts

to solve this problem, are given in WO-A-9309093, WO-A-9426707 and WO-A-9525718, the contents of which are incorporated herein by reference.

It is currently believed that such vitamin D analogues act as general regulators of cell growth and differentiation through receptor-mediated (especially nuclear receptor-mediated) processes involving modulation of vitamin D responsive genes (M.R. Waters, Endoc. Rev. **13**, pp. 719-764 [1992]). It has also hitherto been assumed that the seco steroid 5,7,10(19)-triene system or a similar 19-nor seco steroid 5,7-diene system is a prerequisite for any form of cell modulating activity. Thus, whilst workers investigating vitamin D analogues have modified the A-ring and 17-position side chain and in certain cases have made more drastic modifications to the overall molecular skeleton such as modification or even elimination of the C- and/or D-rings, they have attempted to retain the triene or conjugated diene system (Gui-Dong Zhu *et al.*, Bioorganic & Med. Chem. Lett. **6**, pp. 1703-1708 [1996]; K. Sabbe *et al.*, Bioorganic & Med. Chem. Lett. **6**, pp. 1697-1702 [1996]).

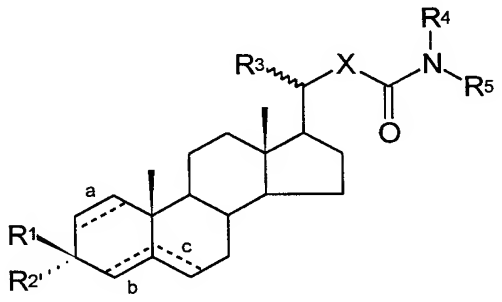
Workers have recently reported the observation of non-genomic rapid responses to vitamin D analogues which they attribute to interaction with a putative cell membrane-located vitamin D receptor (A.W. Norman *et al.*, J. Steroid Biochem. and Mol. Biol. **56**, pp. 13-22 [1996]). It has also been reported that such non-genomic rapid effects may be elicited by 1α,3β,25-trihydroxycholesta-5,7-diene, i.e. the pro-vitamin form of 1α,25-dihydroxy vitamin D_3, which is not a seco steroid; this has been attributed to the ability of the pro-vitamin to mimic the 6,7-s-cis conformation of the normal vitamin D triene (Norman, *op. cit.*). However, the pro-vitamin has been reported to have little ability to elicit the genomic effect believed to underlie modulation of cell growth and differentiation (Norman,

op. cit.) and has also been reported not to exhibit the typical effects of vitamin D on skin (R. Gniadecki *et al.*, British J. Dermatol. **132**, pp. 841-852 [1995]).

The present invention is based on the surprising finding that a range of simple sterol derivatives which have an intact tetracyclic nucleus and thus lack both the seco steroid triene system of vitamin D analogues and the ability to mimic a conjugated conformational isomer thereof, exhibit potent effects on the modulation of cell growth and differentiation as estimated by their ability to inhibit growth and promote differentiation of a variety of cancer cell lines. The compounds possess an advantageous therapeutic ratio by virtue of their low levels of calcaemic activity, for example as determined by their effects on serum calcium and phosphorus levels in rats.

The compounds of the invention comprise 3β-sterols (and O-protected derivatives thereof) having a double bond at the 5(6)-position and an amide-terminated 17-position side chain, as well as corresponding 17-substituted steroid-3-ones having 4-ene or 1,4-diene double bonds.

Thus according to one embodiment of the invention there are provided compounds of formula (I)



(I)

in which:

R^1 represents a hydroxyl group or protected hydroxyl group, R^2 represents a hydrogen atom and a double bond is present at c, or R^1 and R^2 together represent an oxo

group and a double bond is present at b or double bonds are present at a and b;

R^3 represents a methyl group having α- or β-configuration;

R^4 and R^5, which may be the same or different, are selected from hydrogen atoms and aliphatic, cycloaliphatic, araliphatic and aryl groups, or together with the nitrogen atom to which they are attached form a heterocyclic group; and

X represents a polymethylene group containing 2-5 carbon atoms, an oxa group-containing analogue thereof in which a methylene group other than that attached to the $-CO.NR^4R^5$ moiety is replaced by an oxygen atom, or an unsaturated analogue thereof containing up to two double bonds.

Where R^1 represents a protected hydroxyl group this may, for example, comprise any suitable cleavable O-protecting group such as is commonly known in the art. Representative groups include (i) etherifying groups such as silyl groups (e.g. tri(lower alkyl)silyl groups such as trimethylsilyl, triethylsilyl, triisopropylsilyl or t-butyldimethylsilyl; tri(aryl)silyl groups such as triphenylsilyl; and mixed alkyl-arylsilyl groups), lower (e.g. C_{1-6}) alkyl groups optionally interrupted by an oxygen atom (e.g. such as methyl, methoxymethyl or methoxyethoxymethyl) and cyclic ether groups (e.g. such as tetrahydropyranyl), and (ii) esterifying groups such as lower (e.g. C_{1-6}) alkanoyl (e.g. such as acetyl, propionyl, isobutyryl or pivaloyl), aroyl (e.g. containing 7-15 carbon atoms, such as benzoyl or 4-phenylazobenzoyl), lower (e.g. C_{1-6}) alkane sulphonyl (e.g. such as methane sulphonyl or halogenated methane sulphonyl) and arene sulphonyl (e.g. such as p-toluene sulphonyl).

Such O-protected derivatives of compounds of formula (I) are useful in the preparation of active compounds (I) in which R^1 represents a hydroxy group and

may also, where the O-protecting group is metabolically labile *in vivo*, be useful directly in therapy.

Where R^3 in formula (I) is a methyl group in the α-configuration the compounds have the 20R configuration characteristic of natural sterols such as cholesterol; where R^3 is in the β-configuration the compounds have the 20S configuration of the corresponding epi-derivatives. It will be appreciated that the invention also embraces mixtures of the two isomers.

Aliphatic groups represented by R^4 and R^5 may, for example, include lower (e.g. C_{1-6}) alkyl groups such as methyl, ethyl, propyl and butyl groups. Cycloaliphatic groups may, for example, include lower (e.g. C_{3-8}) cycloalkyl groups such as cyclopropyl, cyclopentyl and cyclohexyl groups. Araliphatic groups may, for example, include C_{6-12} aryl-C_{1-4} alkyl groups such as benzyl or phenethyl. Aryl groups may, for example, include C_{6-12} carbocyclic aryl groups such as phenyl or naphthyl, optionally carrying one or more substituents, for example selected from halo (e.g. chloro or bromo), lower (e.g. C_{1-4}) alkyl such as methyl, lower (e.g. C_{1-4}) alkoxy such as methoxy, lower (e.g. C_{2-4}) alkanoyl such as acetyl, lower (e.g. C_{1-4}) alkylamino or dialkylamino such as methylamino or dimethylamino, nitro, carbamoyl and lower (e.g. C_{2-4}) alkanoylamino such as acetamido.

Where the group R^4R^5N- represents a heterocyclic group this will typically contain at least one heteroatom selected from O, N and S, and may comprise one or more rings, e.g. each having 5 or 6 ring members. Representative heterocyclic R^4R^5N- groups thus include N-attached pyrrolyl, pyrazolyl, imidazolyl, indolyl, indazolyl, purinyl, pyrrolindinyl, imidazolidinyl, pyrazolidinyl, piperidinyl, morpholino, thiazolidinyl and thiamorpholino.

The group X may, for example, be represented by the formula $-CH_2-(CH=CH)_m-(CH_2)_n-$ where m is 0, 1 or 2 and n is 0 or an integer such that 2m + n = 1, 2, 3 or 4.

Alternatively X may be a group of formula $-(CH_2)_p-O-(CH_2)_q-$ where p is 0, 1, 2 or 3, q is 1, 2, 3 or 4 and p + q does not exceed 4.

The cell modulating activity of compounds according to the invention, including O-protected derivatives in which the O-protecting group is metabolically labile, combined with their substantial lack of calcaemic effect, render them of interest both alone and as adjuncts in the management of neoplastic disease, particularly myelogenous leukemias as well as neoplastic disease of the brain, breast, stomach, gastrointestinal tract, prostate, pancreas, uro-genital tract (male and female) and pulmonary neoplasia. Their ability to promote closure of mouse ear punches suggests their use, either alone or as adjuncts, as agents to promote wound healing. They may also be useful, either alone or as adjuncts, in the chemotherapy of infection and in other therapeutic modalities in which mononuclear phagocytes are involved, for example in treatment of bone disease (e.g. osteoporosis, osteopenia and osteodystrophy as in rickets or renal osteodystrophy), autoimmune disease, host-graft reaction, transplant rejection, inflammatory diseases (including modulation of immunoinflammatory reactions), neoplasias and hyperplasias, myophathy, enteropathy and spondylitic heart disease. Additionally, they may be useful in suppression of parathyroid hormone (e.g. as in serum calcium homeostasis), in treatment of dermatological diseases (for example including acne, alopecia, eczema, pruritus, psoriasis and skin aging, including photoaging), hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment and senile dementia (including Alzheimer's disease), in fertility control in both human and animal subjects, and in management of disorders involving blood clotting (e.g. by dissolution of existing clots and/or by prevention of clotting). The invention embraces use

of these compounds in the therapy or prophylaxis of such conditions and in the manufacture of medicaments for use in such treatment or prophylaxis.

Active compounds according to the invention may be formulated for administration by any convenient route, e.g. orally (including sublingually), parenterally, rectally or by inhalation; pharmaceutical compositions so formulated comprise a feature of the invention.

Orally administrable compositions may, if desired, contain one or more physiologically compatible carriers and/or excipients and may be solid or liquid. The compositions may take any convenient form including, for example, tablets, coated tablets, capsules, lozenges, aqueous or oily suspensions, solutions, emulsions, syrups, elixirs and dry products suitable for reconstitution with water or another suitable liquid vehicle before use. The compositions may advantageously be prepared in dosage unit form. Tablets and capsules according to the invention may, if desired, contain conventional ingredients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth or polyvinyl-pyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. Tablets may be coated according to methods well known in the art.

Liquid compositions may contain conventional additives such as suspending agents, for example sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethylcellulose, carboxymethylcellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate or acacia; non-aqueous vehicles, which may include edible oils, for example vegetable oils such as arachis oil, almond oil, fractionated coconut oil, fish-

liver oils, oily esters such as polysorbate 80, propylene glycol, or ethyl alcohol; and preservatives, for example methyl or propyl p-hydroxybenzoates or sorbic acid. Liquid compositions may conveniently be encapsulated in, for example, gelatin to give a product in dosage unit form.

Compositions for parenteral administration may be formulated using an injectable liquid carrier such as sterile pyrogen-free water, sterile peroxide-free ethyl oleate, dehydrated alcohol or propylene glycol or a dehydrated alcohol/propylene glycol mixture, and may be injected intravenously, intraperitoneally or intramuscularly.

Compositions for rectal administration may be formulated using a conventional suppository base such as cocoa butter or another glyceride.

Compositions for administration by inhalation are conveniently formulated for self-propelled delivery, e.g. in metered dose form, for example as a suspension in a propellant such as a halogenated hydrocarbon filled into an aerosol container provided with a metering dispense valve.

It may be advantageous to incorporate an antioxidant, for example ascorbic acid, butylated hydroxyanisole or hydroquinone in the compositions of the invention to enhance their storage life.

Where any of the above compositions are prepared in dosage unit form these may for example contain 0.2-2500 μg, e.g. 0.4-500 μg, of active compound according to the invention per unit dosage form. The compositions may if desired incorporate one or more further active ingredients.

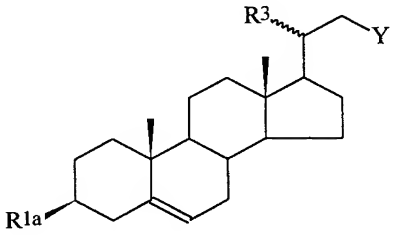
A suitable daily dose of an active compound according to the invention may for example be in the range 0.4-5000 μg, e.g. 0.8-1000 μg, per day, depending on factors such as the severity of the condition being treated and the age, weight and condition of the

subject.

Compounds according to the invention may be prepared by any convenient method, for example by reaction of a compound containing a precursor for the desired side chain in one or more stages and with one or more reactants serving to form the desired 17-position side chain, followed if necessary and/or desired by removal of any O-protecting group, oxidation of a 3β-ol to a 3-one and consequent isomerisation of a 5(6)-ene to a 4-ene, and oxidation to form a 1,4-diene.

Appropriate techniques for formation of a desired side chain include those described in the aforementioned WO-A-9309093 and WO-A-9426707.

By way of example, compounds of formula (I) in which X is a group $-CH_2-(CH=CH)_m-(CH_2)_n-$ as hereinbefore defined may be prepared by appropriate reaction of a compound of formula (II)



(II)

in which:

R^{1a} represents a protected hydroxyl group,

R^3 is as hereinbefore defined, and

Y represents an oxo or phosphoranylidene group; a metallated silane or sulphone group; a group $-(CH_2)_xL$ where x is 0, 1 or 2 and L represents a leaving group (e.g. a sulphonate ester group such as lower alkyl sulphonyloxy, lower fluoroalkyl sulphonyloxy or aryl sulphonyloxy, or a halogen atom such as chlorine, bromine or iodine); or a group $-(CH_2)_yR^6$ where y is 0, 1, 2 or 3 and R^6 represents a cyano group or an esterified carboxyl or thiocarboxyl group (e.g. an alkoxycarbonyl,

aralkoxycarbonyl, aryloxycarbonyl, alkylthiocarbonyl, aralkylthiocarbonyl or arylthiocarbonyl group).

Reactions which may be used to prepare compounds of formula (I) in which X represents a polymethylene group (i.e. where m = 0) include:-

(1) Reaction of a compound of formula (II) in which Y represents a group $-(CH_2)_xL$ as hereinbefore defined with a metallated or dimetallated salt of an amide of formula (III)

$$CH_3.CO.NR^4R^5 \qquad (III)$$

(where R^4 and R^5 are as hereinbefore defined). Representative salts include alkali metal salts such as lithium salts and may be prepared by reaction with a base such as lithium diisopropylamide.

(2) Reaction of a compound of formula (III) in which Y represents a group $-(CH_2)_yR^6$ as hereinbefore defined to convert the ester, thioester or cyano group R^6 to the desired amide group, e.g. directly by aminolysis of an ester or thioester or indirectly via the corresponding free acid obtained by hydrolysis of the ester, thioester or nitrile or via an acid halide obtained therefrom. It will be appreciated that nitriles of formula (II) may be partially hydrolysed so as directly to yield compounds (I) in which R^4 and R^5 are hydrogen atoms.

(3) Reaction of a compound of formula (II) in which Y represents a group $-(CH_2)_xL$ as hereinbefore defined with a reagent such as a metal cyanide or metallated trithiane which is capable of introducing a one carbon fragment, and conversion of the group so introduced into the desired $-CO.NR^4R^5$ group, for example as described for process (2).

Reactions which may be used to prepare compounds of formula (I) in which X is unsaturated (i.e. where m = 1 or 2) include:-

(4) Reaction of a compound of formula (II) in which Y represents an oxo group according to a Wittig type reaction, for example with a phosphorane of formula (IV)

$$(R^h)_3P=CH-(X^1)_z-R^c \qquad (IV)$$

where X^1 is an alkylene or alkenylene group containing up to 2 carbon atoms; z is 0 or 1; R^h is a hydrocarbyl group (e.g. an alkyl or aralkyl group or an aryl group such as phenyl); and R^c is the carbamoyl group $-CO.NR^4R^5$ as hereinbefore defined or a precursor group convertible thereto (e.g. an ester, thioester or cyano group). Where R^c represents a precursor group, the reaction is followed by conversion to generate the group $-CO.NR^4R^5$, for example as described for process (2). Alternatively the phosphorane (IV) may be replaced by a metallated silane (V)

$$(R^h)_3Si-CHM-(X^1)_z-R^c \qquad (V)$$

or a metallated sulphone (VI)

$$R^hSO_2-CHM-(X^1)_z-R^c \qquad (VI)$$

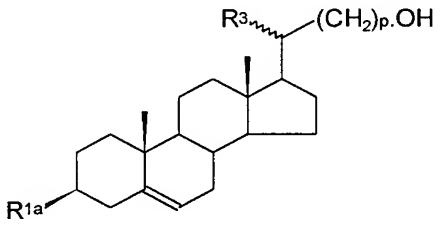
where X^1, z, R^h and R^c are as hereinbefore defined and M represents a metal atom (e.g. an alkali metal such as lithium or sodium). In this last case the reaction is immediately followed by reduction of the intermediate hydroxysulphone to form the required double bond, for example using sodium amalgam. It will be appreciated that reactions of this type may also be effected using a compound of formula (II) in which Y is a phosphoranylidene group $=P(R^h)_3$ or a metallated derivative of a compound (II) in which Y is $-Si(R^h)_3$ or $-SO_2R^h$ with an aldehyde of formula (VII)

$$OHC-(X^1)_z-R^c \qquad (VII)$$

(R^h, X^1, z and R^c having the above-defined meanings).

Compounds of formula (I) wherein X is a group $-(CH_2)_p-O-(CH_2)_q-$ as hereinbefore defined may, for example, be prepared by:-

(5) Reaction of a compound of formula (VIII)



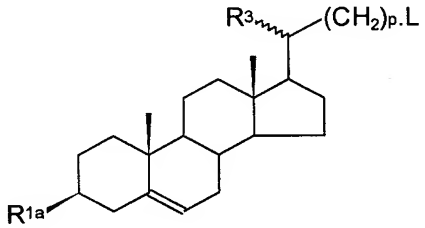
(VIII)

(where R^{1a}, R^3 and p are as hereinbefore defined) with a compound of formula (IX)

$$L.(CH_2)_q.R^c \qquad (IX)$$

(where R^c, L and q are as hereinbefore defined, L preferably being a halogen atom), followed if necessary by conversion of a precursor group R^c to generate the desired group $-CO.NR^4R^5$, e.g. as described above for process (2).

(6) Reaction of a compound of formula (X)



(X)

(where R^{1q}, R^3, L and p are as hereinbefore defined, L preferably being a highly reactive leaving group such as trifluoroacetate, tosylate or trifluoromethane sulphonate) with a compound of formula (XI)

$$HO.(CH_2)_q.R^c \qquad (XI)$$

(where R^c and q are as hereinbefore defined), followed if necessary by conversion of a precursor group R^c to generate the desired group $-CO.NR^4R^5$, e.g. as described above.

(7) Where q is 2, by base catalysed Michael addition of a compound of formula (VIII) as defined above to an acrylate ester, e.g. of formula (XII)

$$CH_2=CH.CO.OR^e \qquad (XII)$$

(where R^e is an esterifying group, e.g. a hydrocarbyl group such as a lower alkyl or aryl group), followed by conversion of the ester grouping to the desired group $-CO.NR^4N^5$, e.g. as described above.

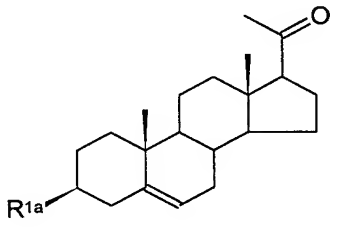
Reagents such as compounds of formula (IX) in which R^c is the carbamoyl group $-CO.NR^4R^5$ may, for example, be prepared by reaction of an appropriate ω-haloalkanoyl chloride (e.g. 4-bromobutyryl chloride where it is desired to synthesise a compound of the invention in which q is 3) with an amine R^4R^5NH (where R^4 and R^5 are as hereinbefore defined). It is convenient to prepare such a reagent *in situ*, i.e. without subsequent purification, preferably using a molar excess of the amine so as to leave a sufficient excess of base to react with acid liberated in the ensuing coupling reaction with a compound of formula (VIII).

Conversion of the protected hydroxyl group R^{1a} in a product to a hydroxyl group R^1 may, for example, be effected by conventional deprotection methods such as are well documented in the literature. Thus an esterifying protecting group may be removed by basic hydrolysis, for example using an alkali metal alkoxide in an alkanol. Etherifying protecting groups such as silyl groups may be removed by acid hydrolysis or by treatment with a fluoride salt, for example a tetraalkylammonium fluoride such as tetrabutylammonium fluoride. It will be appreciated that the use of acid-

labile but base-stable silyl protecting groups may be of particular advantage during homologation steps to build up a desired 17-position side chain in view of the strongly basic conditions normally employed for such reactions.

Conversion of 3β-ols of formula (I) to corresponding 3-ones may be effected using any appropriate oxidising agent, e.g. Swern oxidation; the oxidation will normally be accompanied by spontaneous isomerisation to the 4-en-3-one. Where a 1,4-dien-3-one is desired, the additional double bond may, for example, be generated by reaction with selenium dioxide in t-butanol, or by dehydrogenation using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

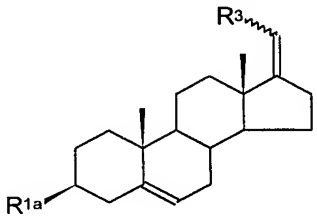
Starting materials of formula (II) in which Y represents oxo may be prepared from known pregnenolones (XIII)



(XIII)

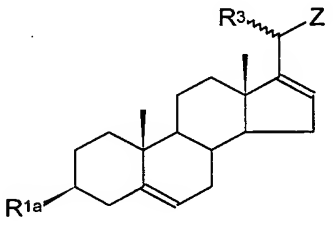
(where R^{1a} is as hereinbefore defined) by Wittig reaction with an alkoxymethylenephosphorane or other one carbon atom alkoxy ylide.

Alternatively, known steroid-5(6)-en-17-ones may be subjected to a Wittig reaction to generate a compound of formula (XIV)



(XIV)

(where R^{1a} and R^3 are as hereinbefore defined) which may then be reacted with a dienophile (e.g. formaldehyde or a functional equivalent thereof, or a proparagyl ester) to yield a compound of formula (XV)



(XV)

(where R^{1a} and R^3 are as hereinbefore defined and Z is either $-CH_2OH$ or $-CH=CH.CO.OR^h$ where R^h is as hereinbefore defined). Where Z is $-CH_2OH$ this may be converted to a $-CH_2Y$ group, for example, by oxidation to form a compound in which Y is oxo, or by sulphonate ester formation (e.g. tosylation), and preferably also nucleophilic displacement with halide ion, to yield a compound in which Y is $-(CH_2)_xL$ where x is 0. The 16,17-double bond is easily reduced and may be removed by hydrogenation at any appropriate step of the reaction sequence.

Preparation of the above starting materials and other intermediates useful in the preparation of compounds according to the invention is described by Batcho *et al.*, Helv. Chim. Acta. **64**, pp. 1682-1687 [1981], Midland *et al.*, Tetrahedron Lett. **23(20)**, pp. 2077-2080 [1982], Krubiner *et al.*, J. Org. Chem. **31**, pp. 24-26 [1965] and Dauben *et al.*, J. Am. Chem. Soc. **103**, pp. 237-238 [1980].

The following non-limitative examples serve to illustrate the invention.

Preparation 1

3β-Triisopropylsilyloxypregn-5(6)-ene-20-carboxaldehyde [Formula (II) - R^{1a} = $(i\text{-}Pr)_3SiO$, R^3 = CH_3, Y = O]

A solution of methoxymethyl-triphenylphosphonium chloride (9.87 g) in a mixture of tetrahydrofuran (50 ml) and toluene (50 ml) at 0°C was treated with lithium diisopropylamide (14.46 ml of a 1.5 M solution in tetrahydrofuran). After 30 minutes 3β-triisopropylsilyloxypregn-5(6)-en-20-one (6.3 g) in toluene (4 ml) followed by a toluene wash (2 ml) were added dropwise, and the resulting mixture was stirred at 0°C for 1 hour, allowed to warm to room temperature and stored overnight with stirring. The mixture was then treated with ammonium chloride and extracted with ethyl acetate to give the intermediate 20-methoxymethylidene compound, which was purified by chromatography: NMR ($CDCl_3$) δ 0.63 (18-H's), 3.33 (OCH_3), 5.6 (6-H).

The above intermediate (all) was taken up in a mixture of acetic acid (54 ml), water (2.4 ml) and tetrahydrofuran (27 ml), treated with *p*-toluenesulphonic acid (240 mg) and stored overnight with stirring. The product was extracted into ethyl acetate and washed with aqueous sodium bicarbonate, whereafter solvent removal gave the 3-desilylated analogue of the title compound (4.23 g): NMR ($CDCl_3$) δ 0.63, 0.72 (two signals, 18-H's), 1.0 (19,20-H's), 5.23 (6-H), 10.7 (HC=O); IR ($CDCl_3$) ν_{max} 1770 cm^{-1}.

The desilylated intermediate (all) in methylene chloride (8.5 ml) containing imidazole (2.415 g) was treated with triisopropylsilyl chloride (1.86 ml), and the resulting mixture was stored overnight at room temperature with stirring. The product was extracted into ethyl acetate, washed with water and isolated by column chromatography

to give the title compound.

Preparation 2

20α- and 20β-hydroxymethyl-3β-triisopropylsilyloxypregn-5(6)-ene [Formula (II) - R^{1a} = $(i\text{-}Pr)_3SiO$, R^3 = CH_3, Y = OH]

A solution of the 20-carboxaldehyde from Preparation 1 (1.2 g) in a mixture of methanol (12 ml) and benzene (1.2 ml) was treated with sodium borohydride (800 mg), stirred at room temperature for 1 hour, cooled, treated with ammonium chloride and extracted with ethyl acetate. Solvent removal from the extract afforded the title compounds, which were resolved by chromatography into less polar and more polar isomers, tentatively assigned the 20α- and 20β-configurations respectively.

Preparation 3

20α-Tosyloxymethyl-3β-triisopropylsilyloxypregn-5(6)-ene [Formula (II) - R^{1a} = $(i\text{-}Pr)_3SiO$, R^3 = β-CH_3, Y = OTs]

The more polar alcohol from Preparation 2 (310 mg) in methylene chloride containing pyridine (0.355 ml) was treated with tosyl chloride (243 mg), stirred at room temperature for 3 hours, treated with aqueous sodium bicarbonate and methylene chloride, stirred overnight and treated with 1,8-bisdimethylaminonaphthalene (25 mg). The product was extracted into methylene chloride and the extracts were washed successively with 2% hydrochloric acid, sodium bicarbonate and water, dried and concentrated *in vacuo*. Chromatography gave the title compound (380 mg).

Preparation 4

20β-Bromomethyl-3β-triisopropylsilyloxypregn-5(6)-ene [Formula (II) - R^{1a} = $(i\text{-}Pr)_3SiO$, R^3 = β-CH_3, Y = Br]

The tosylate from Preparation 3 (380 mg) was dissolved in a mixture of acetonitrile (12 ml) and 1,2-dichloroethane (12 ml) containing 1,8-bisdimethylaminonaphthalene (33 mg), treated with lithium bromide (621 mg) and heated under reflux with stirring for 3 hours. The product was extracted into methylene chloride, washed and purifed by chromatography to give the title compound: NMR ($CDCl_3$) δ 0.66 (18-H's), 5.06 (6-H).

Example 1

a) 3β-Triisopropylsilyloxy-20-epi-chol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = $(i\text{-}Pr)_3SiO$, R^2 = H, R^3 = β-CH_3, $R^4 + R^5$ = $(CH_2)_5$, X = $(CH_2)_2$, double bond at c]

A solution of lithium diisopropylamide (3 ml of a 2M solution in tetrahydrofuran) in tetrahydrofuran (10 ml) was cooled to -78°C. N-Acetylpiperidine (914 mg) in tetrahydrofuran (1 ml and 1 ml wash) was added and the mixture was brought to room temperature for 1 hour and then cooled again to -78°C. Two thirds of the mixture was removed and the bromide from Preparation 4 (107 mg) in tetrahydrofuran (1 ml and 1 ml wash) was added to the remainder. Hexamethylphosphoramide was then added and the resulting mixture was stirred at -78°C for 1 hour and overnight at room temperature. After treatment with ammonium chloride the product was extracted into ethyl acetate, washed, dried and purified by chromatography to give the title compound (120 mg): NMR ($CDCl_3$) δ 0.63 (18-H's), 3.30-3.2 (m, N-CH_2's), 5.03 (6-H); IR ($CDCl_3$) ν_{max} 1620, 1440 cm^{-1}.

b) 3β-Hydroxy-20-epi-chol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = β-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)_2$, double bond at c]

The product from (a) above (120 mg) in tetrahydrofuran (1 ml) was treated with tetrabutylammonium fluoride (1 ml of a 1M solution in tetrahydrofuran) and allowed to stand with stirring at room temperature overnight. The product was extracted into methylene chloride, washed with water and purified by chromatography to give the title compound (88 mg): NMR ($CDCl_3$) δ 0.66 (18-H's), 0.93 (19,21-H's), 1.53 (3-5 H's of piperidine ring), 3.26-3.2 (m, N-CH_2's), 5.06 (6-H); IR ($CDCl_3$) ν_{max} 3400, 1620, 1440 cm^{-1}.

Example 2

3-Oxo-20-epi-chol-4-enic acid, piperidine amide [Formula (I) - R^1 + R^2 = O, R^3 = β-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)_2$, double bond at b]

A solution of aluminium isopropoxide (96 mg) in toluene (2.5 ml) was added dropwise to a refluxing solution of the product from Example 1(b) (80 mg) in toluene (4.8 ml) containing cyclohexanone (0.5 ml). Heating was continued for 2 hours, whereafter the mixture was cooled and the product was extracted into ethyl acetate and purified by chromatography to give the title compound (46 mg): NMR ($CDCl_3$) δ 0.66 (18-H's), 0.93 (21-H's), 1.1 (19-H's), 3.63 (m, N-CH_2's), 5.46 (4-H); IR ($CDCl_3$) ν_{max} 1660, 1620, 1440 cm^{-1}.

Example 3

a) 3β-Triisopropylsilyloxychol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = $(i\text{-}Pr)_3SiO$, R^2 = H, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)_2$, double bond at c]

Treatment of the less polar alcohol from Preparation 2 in accordance with the procedures of Preparations 3 and 4 and Example 1(a) afforded the title compound: NMR ($CDCl_3$) δ 0.66 (18-H's), 3.56 (m, N-CH_2's), 5.03 (6-H); IR ($CDCl_3$) ν_{max} 1620, 1440 cm^{-1}.

b) 3β-Hydroxychol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)_2$, double bond at c]

The product from (a) above was treated according to the procedure of Example 1(b) to give the title compound: NMR ($CDCl_3$) δ 0.66 (18-H's), 0.96 (19,21-H's), 1.6 (3-5 H's of piperidine ring), 3.3 (m, N-CH_2's), 5.1 (6-H); IR ($CDCl_3$) ν_{max} 3600, 1620, 1440 cm^{-1}.

Example 4

3-Oxochol-4-enic acid, piperidine amide [Formula (I) - R^1 + R^2 = O, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)_2$, double bond at b]

The product from Example 3(b) was treated according to the procedure of Example 2 to give the title compound: NMR ($CDCl_3$) δ 0.7 (18-H's), 1.1, 1.21 (19,21-H's), 3.26 (m, N-CH_2's), 5.33 (4-H); IR ($CDCl_3$) ν_{max} 1660, 1620 cm^{-1}.

Example 5

3β-Hydroxychol-5(6)-enic acid, morpholine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_2O(CH_2)_2$, X = $(CH_2)_2$, double bond at c]

The procedures of Example 3 were repeated, replacing the N-acetylpiperidine in (a) with N-acetylmorphine, to give the title compound: NMR ($CDCl_3$) δ 0.63 (18-H's), 0.96 (19-H's), 3.2-3.7 (m, morpholine-CH_2's), 5.1 (6-H); IR ($CDCl_3$) ν_{max} 3640-3200, 1620, 1430 cm^{-1}.

Example 6

3-Oxochol-4-enic acid, morpholine amide [Formula (I) - R^1 + R^2 = O, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_2O(CH_2)_2$, X = $(CH_2)_2$, double bond at b]

The product from Example 5 was treated according to the procedure of Example 2 to give the title compound: NMR ($CDCl_3$) δ 0.66 (18-H's), 3.1-3.7 (m, morpholine-CH_2's), 5.5 (4-H).

Example 7

3β-Hydroxychol-5(6)-enic acid, thiamorpholine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_2S(CH_2)_2$, X = $(CH_2)_2$, double bond at c]

The procedures of Example 3 were repeated, replacing the N-acetylpiperidine in (a) with N-acetylthiamorphine, to give the title compound: NMR ($CDCl_3$) δ 0.63 (18-H's), 0.93 (19-H's), 2.3-2.7 (m, thiamorpholine-CH_2's), 3.1-3.8 (m, N-CH2's),5.0-5.3 (b, 6-H); IR ($CDCl_3$) ν_{max} 3640-3100, 1620, 1430 cm^{-1}.

Example 8

3-Oxochol-4-enic acid, thiamorpholine amide [Formula (I) - R^1 + R^2 = O, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_2S(CH_2)_2$, X = $(CH_2)_2$, double bond at b]

The product from Example 7 was treated according to the procedure of Example 2 to give the title compound: NMR ($CDCl_3$) δ 0.66 (18-H's), 1.13 (19-H's), 2.3-2.7 (m, thiamorpholine-CH_2's), 3.4-3.9 (m, N-CH_2's), 5.5 (4-H).

Example 9

3β-Hydroxychol-5(6)-enic acid, diisopropyl amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = α-CH_3, R^4 + R^5 = $CH(CH_3)_2$, X = $(CH_2)_2$, double bond at c]

The procedures of Example 3 were repeated, replacing the N-acetylpiperidine in (a) with N-acetyldiisopropylamine, to give the title compound: NMR ($CDCl_3$) δ 0.63 (18-H's), 0.96 (19-H's), 3.0-3.8 (m, 3-H, N-CH's), 5.0-5.3 (b, 6-H); IR ($CDCl_3$) ν_{max} 3640-3100, 1610, 1440 cm^{-1}.

Example 10

3-Oxochol-4-enic acid, diisopropyl amide [Formula (I) - R^1 + R^2 = O, R^3 = α-CH_3, R^4 + R^5 = $CH(CH_3)_2$, X = $(CH_2)_2$, double bond at b]

The product from Example 9 was treated according to the procedure of Example 2 to give the title compound: NMR ($CDCl_3$) δ 0.7 (18-H's), 1.17 (19-H's), 3.0-4.0 (m, N-CH's), 5.57 (s, 4-H).

Example 11

3β-Hydroxy-24,24a-bishomo-chol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = α-CH_3, $R^4 + R^5 = (CH_2)_5$, X = $(CH_2)_4$, double bond at c]

The title compound is prepared from 3β-triisopropylsilyloxychol-5(6)-enic acid by reduction with lithium aluminium hydride followed by the procedures of Example 3.

Example 12

3-Oxo-24,24a-bishomo-chol-4-enic acid, piperidine amide [Formula (I) - $R^1 + R^2$ = O, R^3 = α-CH_3, $R^4 + R^5 = (CH_2)_5$, X = $(CH_2)_4$, double bond at c]

The product from Example 11 is treated according to the procedure of Example 2 to give the title compound.

Example 13

3β-Hydroxy-20-epi-24-homo-22-oxachol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = β-CH_3, $R^4 + R^5 = (CH_2)_5$, X = $O(CH_2)_2$, double bond at c]

A mixture of 3β-triisopropylsilyloxypregn-5(6)-en-20β-ol (390 mg), ethyl acrylate (2.3 ml), sodium hydroxide (9.2 ml, 50% aqueous), tetrabutylammonium hydroxide (.038 ml, 40% aqueous solution) and toluene (23 ml) was stirred at room temperature overnight, then diluted with ether and washed with water then brine. The organic portion was concentrated *in vacuo* and the product (3β-triisopropylsilyloxy-20-epi-24-homo-22-oxachol-5(6)-enic acid, ethyl ester) was isolated by chromatography.

This ester (60 mg) in hexane (6 ml) at -78°C was treated

(dropwise addition) with a solution of piperidyl tin N,N-bistrimethylsilylamide [prepared by reaction of tin bis(N,N-bistrimethylsilylamide) (264 mg) in hexane (6 ml) with piperidine (51 mg)]. The reaction mixture was brought to room temperature, diluted with ethyl acetate, then washed successively with 5M potassium fluoride and brine, dried and concentrated *in vacuo*. The 3-triisopropylsilyl ether of the title product (20 mg) was isolated by chromatography: NMR ($CDCl_3$) δ 0.63 (18-H's), 3.0-3.9 (m, 3-H, 20-H, -O-CH's, N-CH's), 4.9-5.2 (b, 6-H); IR ($CDCl_3$) ν_{max} 1640, 1470 cm^{-1}. Desilylation as in Example 1(b) afforded the title compound: NMR ($CDCl_3$) δ 0.66 (18-H's), 1.0 (19-H's), 3.1-4.0 (m, 3-H, 20-H, -O-CH's, N-CH's), 5.2-5.5 (b, 6-H); IR ($CDCl_3$) ν_{max} 3640-3300, 1620, 1445 cm^{-1}.

Example 14

3β-Hydroxy-20-epi-22-oxachol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = β-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $O(CH_2)$, double bond at c]

A solution of 18-crown-6 (264 mg) in tetrahydrofuran was added dropwise to a mixture of 3β-triisopropylsilyloxypregn-5(6)-en-20β-ol (474 mg) and potassium hydride (0.3 ml of a 35 wt. % dispersion in mineral oil) in tetrahydrofuran (1 ml). The resulting mixture was stirred for 30 minutes at room temperature, cooled to -10°C, then treated (dropwise addition) with N-α-bromoacetylpiperidine (0.5 ml) in tetrahydrofuran (1 ml). After 30 minutes the reaction mixture was brought to room temperature and allowed to stand overnight. The reaction mixture was then quenched by addition of saturated aqueous ammonium chloride, the products were extracted into ether which was then washed with water and brine, and the solvents were removed *in vacuo*. The 3-triisopropylsilyl ether of the title product (360 mg)

was isolated by chromatography: NMR ($CDCl_3$) δ 0.7 (18-H's), 3.1-3.6 (m, 3-H, 20-H, N-CH's), 3.83 (s, -O-CH_2-C=O), 4.9-5.3 (b, 6-H); IR ($CDCl_3$) ν_{max} 1640, 1450 cm^{-1}.

Desilylation according to the procedure of Example 1(b) afforded the title compound: NMR ($CDCl_3$) δ 0.7 (18-H's), 1.0 (19-H's), 3.2-3.7 (m, 3-H, 20-H, N-CH's), 3.9-4.1 (d, -O-CH_2-C=O), 5.1-5.4 (b, 6-H); IR ($CDCl_3$) ν_{max} 3640-3300, 1640, 1450 cm^{-1}.

Example 15

3-Oxo-20-epi-22-oxachol-4-enic acid, piperidine amide [Formula (I) - R^1 + R^2 = O, R^3 = β-CH_3, R^4 + R^5 = $(CH_2)_5$, X = O(CH_2), double bond at b]

The product from Example 14 was oxidised according to the procedure of Example 2 to afford the title compound: NMR ($CDCl_3$) δ 0.7 (18-H's), 1.1 (19-H's), 3.0-3.5 (m, 3-H, 20-H, N-CH's), 3.7-4.0 (d, -O-CH_2-C=O), 5.43 (b, 6-H); IR ($CDCl_3$) ν_{max} 1640, 1450 cm^{-1}.

Example 16

3β-Hydroxychol-5(6),22-dienic acid, piperidine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_5$, X = CH=CH, double bond at c]

The aldehyde from Preparation 1 is converted into the corresponding 5(6),22-unsaturated cholenic acid ethyl ester by reaction with the triphenylphosphoranylidene derivateive of ethyl acetate [Formula (IV) - R^c = $CO.OC_2H_5$, R^h = C_6H_5, z = 0], and the latter is in turn converted into the title compound by reaction with the tin reagent of Example 13, followed by desilylation according to the procedure of Example 1(b).

Example 17

3-Oxo-20-epi-chol-1,4-dienic acid, piperidine amide [Formula (I) - R^1 + R^2 = O, R^3 = β-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)_2$, double bonds at a and b]

The title compound is prepared by dehydrogenating the product from Example 2 with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Example 18

3-Oxochol-1,4-dienic acid, piperidine amide [Formula (I) - R^1 + R^2 = O, R^3 = α-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)_2$, double bonds at a and b]

The title compound is prepared by dehydrogenating the product from Example 4 with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Example 19

3β-Hydroxy-20-epi-24-homo-23-oxachol-5(6)-enic acid, piperidine amide [Formula (I) - R^1 = OH, R^2 = H, R^3 = β-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)O(CH_2)$, double bond at c]

The title compound is prepared from the more polar product of Preparation 2 by following the procedure of Example 14.

Example 20

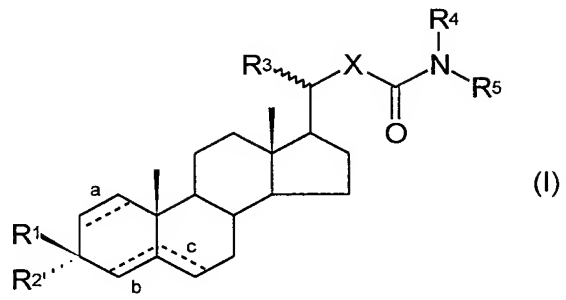
3-Oxo-20-epi-24-homo-23-oxachol-4-enic acid, piperidine amide [Formula (I) - R^1 + R^2 = O, R^3 = β-CH_3, R^4 + R^5 = $(CH_2)_5$, X = $(CH_2)O(CH_2)$, double bond at b]

The title compound is prepared by oxidation of the

product from Example 19 following the procedure of Example 2.

Claims

1. Compounds of formula (I)



(I)

in which:

R^1 represents a hydroxyl group or protected hydroxyl group, R^2 represents a hydrogen atom and a double bond is present at c, or R^1 and R^2 together represent an oxo group and a double bond is present at b or double bonds are present at a and b;

R^3 represents a methyl group having α- or β-configuration;

R^4 and R^5, which may be the same or different, are selected from hydrogen atoms and aliphatic, cycloaliphatic, araliphatic and aryl groups, or together with the nitrogen atom to which they are attached form a heterocyclic group; and

X represents a polymethylene group containing 2-5 carbon atoms, an oxa group-containing analogue thereof in which a methylene group other than that attached to the $-CO.NR^4R^5$ moiety is replaced by an oxygen atom, or an unsaturated analogue thereof containing up to two double bonds.

2. Compounds of formula (I) as claimed in claim 1 wherein R^1 represents a hydroxyl group or a hydroxyl group substituted with a metabolically labile O-protecting group.

3. Compounds of formula (I) as claimed in claim 1 or

claim 2 wherein R^4 and R^5 are selected from hydrogen atoms, C_{1-6} alkyl groups, C_{3-8} cycloalkyl groups, C_{6-12} aryl-C_{1-4} alkyl groups and optionally substituted C_{6-12} carbocyclic aryl groups.

4. Compounds of formula (I) as claimed in claim 1 or claim 2 wherein R^4R^5N- represents a heterocyclic group comprising one or more 5- and/or 6-membered rings and containing at least one heteroatom selected from O, N and S.

5. Compounds of formula (I) as claimed in claim 4 wherein R^4R^5N- represents a piperidino, morpholino or thiamorpholino group.

6. Compounds of formula (I) as claimed in any of the preceding claims wherein X represents a group of formula $-CH_2-(CH=CH)_m-(CH_2)_n-$ where m is 0, 1 or 2 and n is 0 or an integer such that 2m + n = 1, 2, 3 or 4.

7. Compounds of formula (I) as claimed in any of claims 1 to 5 wherein X represents a group of formula $-(CH_2)_p-O-(CH_2)_q-$ where p is 0, 1, 2 or 3, q is 1, 2, 3 or 4 and p + q does not exceed 4.

8. The compounds:

3β-hydroxy-20-epi-chol-5(6)-enic acid, piperidine amide;

3-oxo-20-epi-chol-4-enic acid, piperidine amide;

3β-hydroxychol-5(6)-enic acid, piperidine amide; and

3-oxochol-4-enic acid, piperidine amide.

9. The compounds:

3β-hydroxychol-5(6)-enic acid, morpholine amide;

3-oxochol-4-enic acid, morpholine amide;

3β-hydroxychol-5(6)-enic acid, thiamorpholine amide;

3-oxochol-4-enic acid, thiamorpholine amide;

3β-hydroxychol-5(6)-enic acid, diisopropyl amide;

3-oxochol-4-enic acid, diisopropyl amide;

3β-hydroxy-24,24a-bishomo-chol-5(6)-enic acid, piperidine amide;

3-oxo-24,24a-bishomo-chol-4-enic acid, piperidine amide;

3β-hydroxy-20-epi-24-homo-22-oxachol-5(6)-enic acid, piperidine amide;

3β-hydroxy-20-epi-22-oxachol-5(6)-enic acid, piperidine amide;

3-oxo-20-epi-22-oxachol-4-enic acid, piperidine amide;

3β-hydroxychol-5(6),22-dienic acid, piperidine amide;

3-oxo-20-epi-chol-1,4-dienic acid, piperidine amide;

3-oxochol-1,4-dienic acid, piperidine amide;

3β-hydroxy-20-epi-24-homo-23-oxachol-5(6)-enic acid, piperidine amide; and

3-oxo-20-epi-24-homo-23-oxachol-4-enic acid, piperidine

amide.

10. Active compounds of formula (I) as claimed in any of the preceding claims for use in wound healing, suppression of parathyroid hormone, fertility control or in therapy or prophylaxis of neoplastic disease, infection, bone disease, autoimmune disease, host-graft reaction, transplant rejection, inflammatory disease, neoplasia, hyperplasia, myopathy, enteropathy, spondylitic heart disease, dermatological disease, hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment or senile dementia in a human or animal subject.

11. The use of an active compound of formula (I) as claimed in any of claims 1 to 9 for the manufacture of a medicament for use in wound healing, suppression of parathyroid hormone, fertility control or in therapy or prophylaxis of neoplastic disease, infection, bone disease, autoimmune disease, host-graft reaction, transplant rejection, inflammatory disease, neoplasia, hyperplasia, myopathy, enteropathy, spondylitic heart disease, dermatological disease, hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment or senile dementia in a human or animal subject.

12. Pharmaceutical compositions comprising an active compound of formula (I) as claimed in any of claims 1 to 9 in admixture with one or more physiologically acceptable carriers or excipients.

13. A method of treatment of a human or animal subject to promote wound healing, to suppress parathyroid hormone, to control fertility or to combat neoplastic disease, infection, bone disease, autoimmune disease,

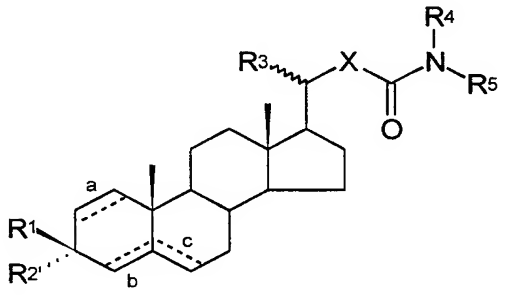
host-graft reaction, transplant rejection, inflammatory disease, neoplasia, hyperplasia, myopathy, enteropathy, spondylitic heart disease, dermatological disease, hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment or senile dementia, comprising administration to said subject of an effective amount of an active compound of formula (I) as claimed in any of claims 1 to 9.

14. A process for the preparation of a compound of formula (I) as defined in claim 1 which comprises reacting a compound containing a precursor for the desired 17-position side chain in one or more stages and with one or more reactants serving to form the desired side chain, followed if necessary and/or desired by removal of any O-protecting group, oxidation of a 3β-ol to a 3-one and consequent isomerisation of a 5(6)-ene to a 4-ene, and oxidation to form a 1,4-diene.

AMENDED CLAIMS

[received by the International Bureau on 17 August 1999 (17.08.99);
original claims 1 and 1-13 amended;
remaining claims unchanged (5 pages)]

1. Compounds of formula (I)



(I)

in which:

R^1 represents a hydroxyl group or protected hydroxyl group, R^2 represents a hydrogen atom and a double bond is present at c, or R^1 and R^2 together represent an oxo group and a double bond is present at b or double bonds are present at a and b;

R^3 represents a methyl group having α- or β-configuration;

R^4 and R^5, which may be the same or different, are selected from hydrogen atoms and aliphatic, cycloaliphatic, araliphatic and aryl groups, or together with the nitrogen atom to which they are attached form a heterocyclic group; and

X represents a polymethylene group containing 2-5 carbon atoms, an oxa group-containing analogue thereof in which a methylene group other than that attached to the $-CO.NR^4R^5$ moiety is replaced by an oxygen atom, or an unsaturated analogue thereof containing up to two double bonds, with the provisos that:

i) when R^3 is α-CH_3 and X is $(CH_2)_2$ then R^4R^5N- does not represent an amino, dimethylamino, diethylamino, imidazolyl or triazolyl group;

ii) when R^3 is α-CH_3 and X is $(CH_2)_3$ then R^4R^5N- is not morpholino; and

iii) when R^3 is α-CH_3 and X is $O(CH_2)_2$ then R^4R^5N is

not dimethylamino.

2. Compounds of formula (I) as claimed in claim 1 wherein R^1 represents a hydroxyl group or a hydroxyl group substituted with a metabolically labile O-protecting group.

3. Compounds of formula (I) as claimed in claim 1 or claim 2 wherein R^4 and R^5 are selected from hydrogen atoms, C_{1-6} alkyl groups, C_{3-8} cycloalkyl groups, C_{6-12} aryl-C_{1-4} alkyl groups and optionally substituted C_{6-12} carbocyclic aryl groups.

4. Compounds of formula (I) as claimed in claim 1 or claim 2 wherein R^4R^5N- represents a heterocyclic group comprising one or more 5- and/or 6-membered rings and containing at least one heteroatom selected from O, N and S.

5. Compounds of formula (I) as claimed in claim 4 wherein R^4R^5N- represents a piperidino, morpholino or thiamorpholino group.

6. Compounds of formula (I) as claimed in any of the preceding claims wherein X represents a group of formula $-CH_2-(CH=CH)_m-(CH_2)_n-$ where m is 0, 1 or 2 and n is 0 or an integer such that 2m + n = 1, 2, 3 or 4.

7. Compounds of formula (I) as claimed in any of claims 1 to 5 wherein X represents a group of formula $-(CH_2)_p-O-(CH_2)_q-$ where p is 0, 1, 2 or 3, q is 1, 2, 3 or 4 and p + q does not exceed 4.

8. The compounds:

3β-hydroxy-20-epi-chol-5(6)-enic acid, piperidine amide;

3-oxo-20-epi-chol-4-enic acid, piperidine amide;

3β-hydroxychol-5(6)-enic acid, piperidine amide; and

3-oxochol-4-enic acid, piperidine amide.

9. The compounds:

3β-hydroxychol-5(6)-enic acid, morpholine amide;

3-oxochol-4-enic acid, morpholine amide;

3β-hydroxychol-5(6)-enic acid, thiamorpholine amide;

3-oxochol-4-enic acid, thiamorpholine amide;

3β-hydroxychol-5(6)-enic acid, diisopropyl amide;

3-oxochol-4-enic acid, diisopropyl amide;

3β-hydroxy-24,24a-bishomo-chol-5(6)-enic acid, piperidine amide;

3-oxo-24,24a-bishomo-chol-4-enic acid, piperidine amide;

3β-hydroxy-20-epi-24-homo-22-oxachol-5(6)-enic acid, piperidine amide;

3β-hydroxy-20-epi-22-oxachol-5(6)-enic acid, piperidine amide;

3-oxo-20-epi-22-oxachol-4-enic acid, piperidine amide;

3β-hydroxychol-5(6),22-dienic acid, piperidine amide;

3-oxo-20-epi-chol-1,4-dienic acid, piperidine amide;

3-oxochol-1,4-dienic acid, piperidine amide;

3β-hydroxy-20-epi-24-homo-23-oxachol-5(6)-enic acid, piperidine amide; and

3-oxo-20-epi-24-homo-23-oxachol-4-enic acid, piperidine amide.

10. Active compounds of formula (I) as defined in any of the preceding claims but not subject to the provisos of claim 1 for use in wound healing, suppression of parathyroid hormone, fertility control or in therapy or prophylaxis of neoplastic disease, infection, bone disease, autoimmune disease, host-graft reaction, transplant rejection, inflammatory disease, neoplasia, hyperplasia, myopathy, enteropathy, spondylitic heart disease, dermatological disease, hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment or senile dementia in a human or animal subject.

11. The use of an active compound of formula (I) as defined in any of claims 1 to 9 but not subject to the provisos of claim 1 for the manufacture of a medicament for use in wound healing, suppression of parathyroid hormone, fertility control or in therapy or prophylaxis of neoplastic disease, infection, bone disease, autoimmune disease, host-graft reaction, transplant rejection, inflammatory disease, neoplasia, hyperplasia, myopathy, enteropathy, spondylitic heart disease, dermatological disease, hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment or senile dementia in a human or animal subject.

12. Pharmaceutical compositions comprising an active compound of formula (I) as defined in any of claims 1 to

9 but not subject to the provisos of claim 1 in admixture with one or more physiologically acceptable carriers or excipients.

13. A method of treatment of a human or animal subject to promote wound healing, to suppress parathyroid hormone, to control fertility or to combat neoplastic disease, infection, bone disease, autoimmune disease, host-graft reaction, transplant rejection, inflammatory disease, neoplasia, hyperplasia, myopathy, enteropathy, spondylitic heart disease, dermatological disease, hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment or senile dementia, comprising administration to said subject of an effective amount of an active compound of formula (I) as defined in any of claims 1 to 9 but not subject to the provisos of claim 1.

14. A process for the preparation of a compound of formula (I) as defined in claim 1 which comprises reacting a compound containing a precursor for the desired 17-position side chain in one or more stages and with one or more reactants serving to form the desired side chain, followed if necessary and/or desired by removal of any O-protecting group, oxidation of a 3β-ol to a 3-one and consequent isomerisation of a 5(6)-ene to a 4-ene, and oxidation to form a 1,4-diene.

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/GB 99/00681

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07J41/00 A61K31/575 C07J43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 562 849 A (LILLY CO ELI) 29 September 1993 see page 100; example 41; table I ---	1-3,6
X	US 4 217 288 A (DELUCA HECTOR F ET AL) 12 August 1980 see column 5, paragraph 2 ---	1-3,6
X	FR 1 512 326 A (ROUSSEL UCLAF) 23 April 1968 see page 3, column 1; example D ---	1,3,6
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 1999	17/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Watchorn, P

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 99/00681

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SHEETS J J ET AL: "Active site-directed inhibitors of cytochrome P-450scc. Structural and mechanistic implications of a side chain-substituted series of amino-steroids" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 258, no. 19, 1983, pages 11446-11452, XP002104638 MD US see page 11447, column 1, paragraph 12 ---	1-3
X	DE 28 14 347 A (DIAMOND SHAMROCK CORP) 5 October 1978 see example 1F ---	1,2,4-6, 9
X	EP 0 200 859 A (ERBA FARMITALIA ;CONSIGLIO NAZIONALE RICERCHE (IT)) 12 November 1986 see example 2 ---	1-3,6
X	MURATO K ET AL: "Photochemical reactions. Part 108. Photochemistry of N-acylazoles. VI. Photoreactivities of 1-acyl-1,2,4-triazoles and of 2-acyltetrazoles" HELVETICA CHIMICA ACTA., vol. 63, no. 3, 1980, pages 588-605, XP002104639 BASEL CH see page 589, compound 10 ---	1,2,4,6
X	CHEMICAL ABSTRACTS, vol. 096, no. 25, 21 June 1982 Columbus, Ohio, US; abstract no. 212834, SKLAR L A ET AL: "Fluorescent cholesteryl esters in the core of low density lipoprotein" page 280; column 1; XP002104646 see abstract & BIOCHEM. BIOPHYS. RES. COMMUN. , vol. 105, no. 2, 1982, pages 674-680, ---	1-3,6
X	IWASAKI S: "Photochemical reactions. Part 92. Photochemistry of imidazolides. II. C2-C3 cleavage of carboxylic acid chains. A convenient new method for the side-chain degradation of bile acids and of lanosterol" HELVETICA CHIMICA ACTA., vol. 59, no. 8, 1976, pages 2753-2764, XP002104640 BASEL CH see page 2756, compound 18 --- -/--	1,2,4,6

1

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 99/00681

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	D. F. LOUW ET AL: "Delta-5-Steroids and Provitamins D with Branched Side Chains. III. Preparation and Reduction of Some delta-5-Steroid-omega-amides" RECUEIL DES TRAVAUX CHIMIQUES DES PAYS-BAS., vol. 73, no. 9/10, September 1954 - October 1954, pages 667-676, XP002104641 AMSTERDAM NL see page 670, compounds Va-e	1-3,6
X	EP 0 619 304 A (CHUGAI PHARMACEUTICAL CO LTD) 12 October 1994 see example 3	1-3,7
X	R. H. LEVIN ET AL: "Steroid Acids and their Transformation Products. IV. Epimeric 24-Phenyl-5-cholen-3(beta),24-diols and Related Compounds" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 70, 1948, pages 2958-2960, XP002104642 DC US see page 2960, column 1, paragraph 5	1,2,6
X	A. F. CHAPLIN ET AL: "The Steroid Series. Part IV. Some Basic Derivatives" JOURNAL OF THE CHEMICAL SOCIETY., no. 4, 1959, pages 3194-3202, XP002104643 LETCHWORTH GB see page 3197, paragraph 3 - paragraph 4	1,2,6
A	WO 93 09093 A (HOLMES MICHAEL JOHN ;RES INST MEDICINE CHEM (US)) 13 May 1993 see the whole document	1-14
A	WO 94 26707 A (HOLMES MICHAEL JOHN ;RES INST MEDICINE CHEM (US); HESSE ROBERT HEN) 24 November 1994 see the whole document	1-14
A	RUAN B ET AL: "An alternative synthesis of 4,4-Dimethyl-5alpha-cholesta-8,14,24 -trien-3beta-ol, an intermediate in sterol biosynthesis and a reported activator of meiosis and of nuclear orphan receptor LXRalpha" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 8, no. 3, 3 February 1998, page 233-236 XP004136854 see page 233, paragraph 1	1-14

-/--

1

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/GB 99/00681

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	JANOWSKI, BETHANY A. ET AL: "An oxysterol signalling pathway mediated by the nuclear receptor LXR.alpha." NATURE., vol. 383, no. 6602, 24 October 1996, pages 728-731, XP002104644 LONDON GB see page 729; figure 2 see page 730, column 2, paragraph 3 see page 731, column 1, paragraph 2 ---	1-14
P,X	JANOWSKI, BETHANY A. ET AL: "Structural requirements of ligands for the oxysterol liver X receptors LXR.alpha. and LXR.beta." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 96, no. 1, January 1999, pages 266-271, XP002104645 WASHINGTON US see page 268, table 1, compound 7 see page 270, column 2, paragraph 2 see page 271, column 1, paragraph 2 ---	1-3,6, 10-14
P,A	WO 98 32444 A (BASS NATHAN M ;ELIAS PETER M (US); HANLEY KAREN (US); UNIV CALIFOR) 30 July 1998 see claims 1-39 -----	1-14

1

INTERNATIONAL SEARCH REPORT

In. ational application No.
PCT/GB 99/00681

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr ial Application No
PCT/GB 99/00681

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0562849 A	29-09-1993	AU 3551493 A	30-09-1993
		BR 9301342 A	05-10-1993
		CA 2092766 A	28-09-1993
		CN 1081682 A	09-02-1994
		FI 931373 A	28-09-1993
		JP 6056670 A	01-03-1994
		MX 9301653 A	28-02-1994
		ZA 9302178 A	26-09-1994
US 4217288 A	12-08-1980	DE 2920092 A	29-11-1979
		FR 2426044 A	14-12-1979
		GB 2021115 A,B	28-11-1979
		GB 2082588 A,B	10-03-1982
		JP 1425293 C	15-02-1988
		JP 54154747 A	06-12-1979
		JP 62028784 B	23-06-1987
		NL 7903929 A	21-11-1979
		DE 2812741 A	05-10-1978
		FR 2384755 A	20-10-1978
		GB 1574685 A	10-09-1980
		JP 53119858 A	19-10-1978
		NL 7803154 A	26-09-1978
FR 1512326 A	23-04-1968	BE 661288 A	20-09-1965
		CH 435266 A	
		DE 1468902 A	23-04-1970
		DK 115110 B	08-09-1969
		FR 3851 M	
		GB 1066301 A	
		GB 1066302 A	
		GB 1066303 A	
		NL 6503558 A	28-09-1965
		SE 320966 B	23-02-1970
		US 3291690 A	13-12-1966
DE 2814347 A	05-10-1978	US 4172076 A	23-10-1979
		GB 1590667 A	03-06-1981
		JP 53141259 A	08-12-1978
		NL 7803581 A	06-10-1979
EP 0200859 A	12-11-1986	JP 61257996 A	15-11-1986
		US 4732897 A	22-03-1988
EP 0619304 A	12-10-1994	DE 69222183 D	16-10-1997
		DE 69222183 T	22-01-1998
		GR 3025468 T	27-02-1998
		HK 1002729 A	11-09-1998
		US 5436401 A	25-07-1995
		AT 157966 T	15-09-1997
		DK 619304 T	16-02-1998
		ES 2107650 T	01-12-1997
		WO 9312083 A	24-06-1993
		JP 6072994 A	15-03-1994
WO 9309093 A	13-05-1993	AT 134366 T	15-03-1996
		AU 664213 B	09-11-1995
		AU 2901492 A	07-06-1993
		CA 2121678 A	13-05-1993

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ıal Application No
PCT/GB 99/00681

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9309093 A		CZ 9401112 A	15-03-1995
		DE 69208478 D	28-03-1996
		DK 614455 T	18-03-1996
		EP 0614455 A	14-09-1994
		ES 2083771 T	16-04-1996
		FI 942114 A	06-05-1994
		GR 3019601 T	31-07-1996
		HU 67140 A	28-02-1995
		JP 7502499 T	16-03-1995
		NO 941683 A	27-06-1994
		NZ 245041 A	28-03-1995
		PL 171580 B	30-05-1997
		SK 52294 A	09-11-1994
		US 5494905 A	27-02-1996
		US 5686435 A	11-11-1997
		ZA 9208572 A	29-06-1993
WO 9426707 A	24-11-1994	AT 169617 T	15-08-1998
		AU 678246 B	22-05-1997
		AU 6683294 A	12-12-1994
		CA 2162272 A	24-11-1994
		CN 1125436 A	26-06-1996
		CZ 9502903 A	12-06-1996
		DE 69412476 D	17-09-1998
		DE 69412476 T	11-03-1999
		EP 0696275 A	14-02-1996
		ES 2122275 T	16-12-1998
		FI 955316 A	22-12-1995
		HU 74165 A	28-11-1996
		JP 8509971 T	22-10-1996
		NO 954426 A	08-01-1996
		NZ 265665 A	26-05-1997
		US 5756733 A	26-05-1998
		ZA 9403162 A	13-04-1995
WO 9832444 A	30-07-1998	AU 5928898 A	18-08-1998